Conformational changes of calmodulin upon Ca^{2+} binding studied with a microfluidic mixer

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Supporting Information

Files in this Data Supplement:

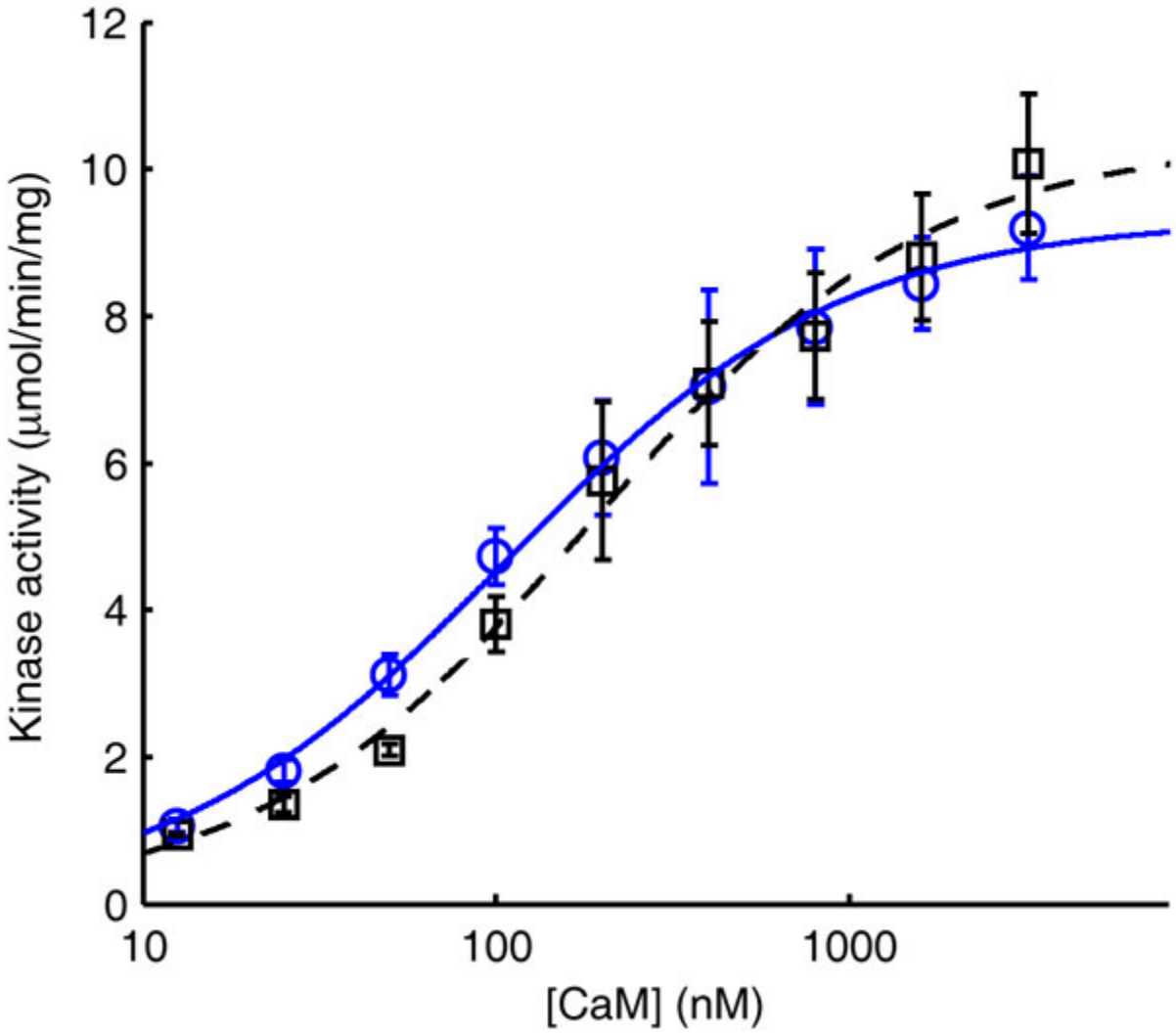

Fig. 7. CaM-kinase II activation by recombinant wild-type CaM (circles) and CaM_{Acr} (squares). The activity of recombinant CaM-kinase II (10 ng per assay) was determined in the presence of increasing concentrations of wtCaM or CaM_{Acr}. The lines represent the best fits of the experimental data to the Hill equation (Eq. **5**) yielding A_{max} (the maximal activity), CaM_{50} (the CaM concentration giving 50% maximal activity), and N (the Hill coefficient). The best fits are obtained with A_{max} = 9.32 ± 0.26, log(CaM_{50}) = -6.97 ± 0.04, and n = 0.91 ± 0.06 for wild-type CaM and A_{max} = 10.4 ± 0.6, log(CaM_{50}) = -6.73 ± 0.08, and n = 0.90 ± 0.11 for CaM_{Acr}. No statistically significant difference was found for any parameters between wtCaM and CaM_{Acr} (p ~ 0.05).

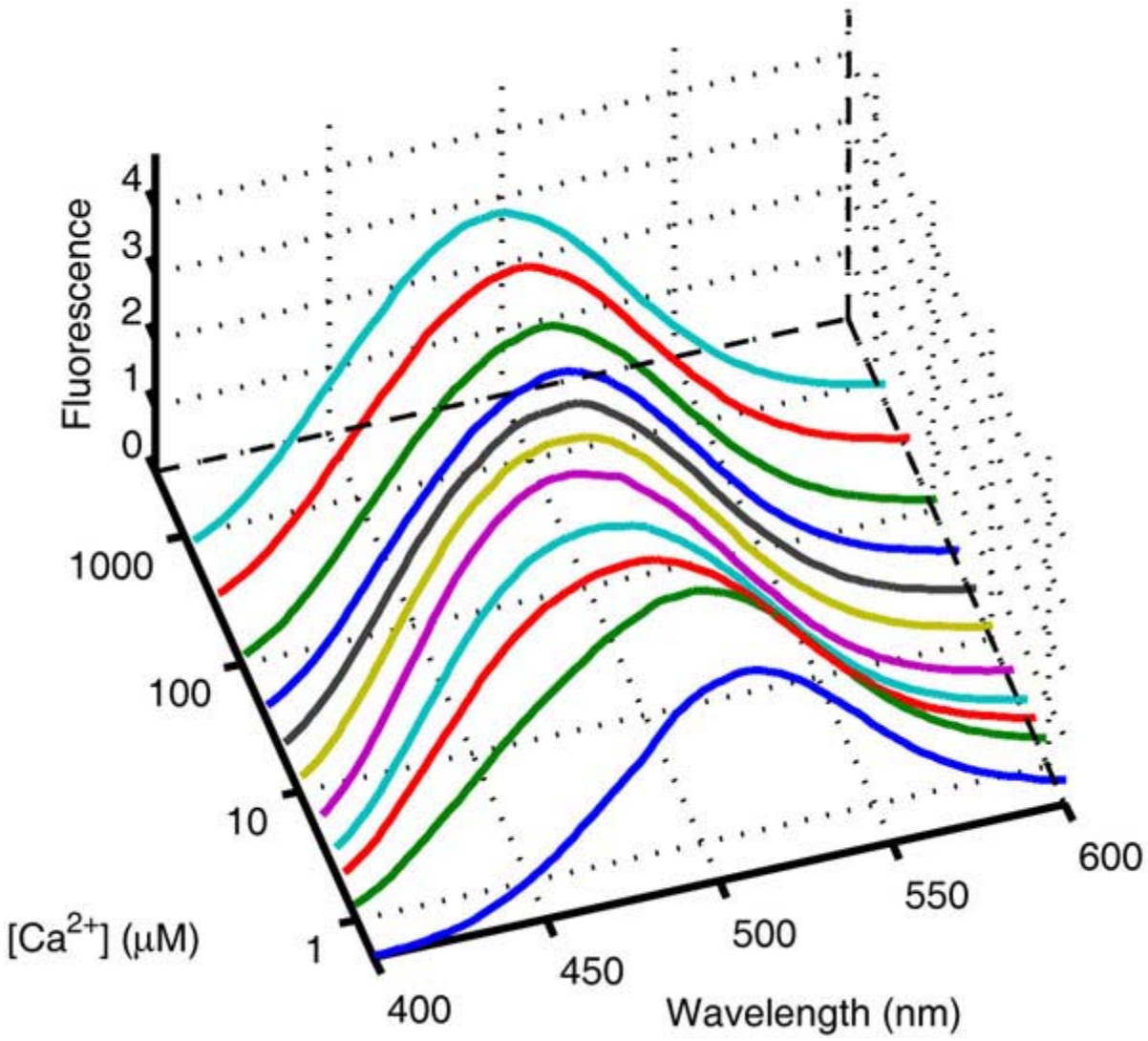

Fig. 8. Emission spectra of CaM_{Acr} at various Ca^{2+} concentrations. Emission from Ca^{2+}-free CaM_{Acr} (bottom curve) was measured by adding 500 mM EGTA. The other curves were measured by titrating CaM_{Acr} in decalcified buffer with $CaCl_2$. The samples were excited at 375 nm at room temperature (~20°C).

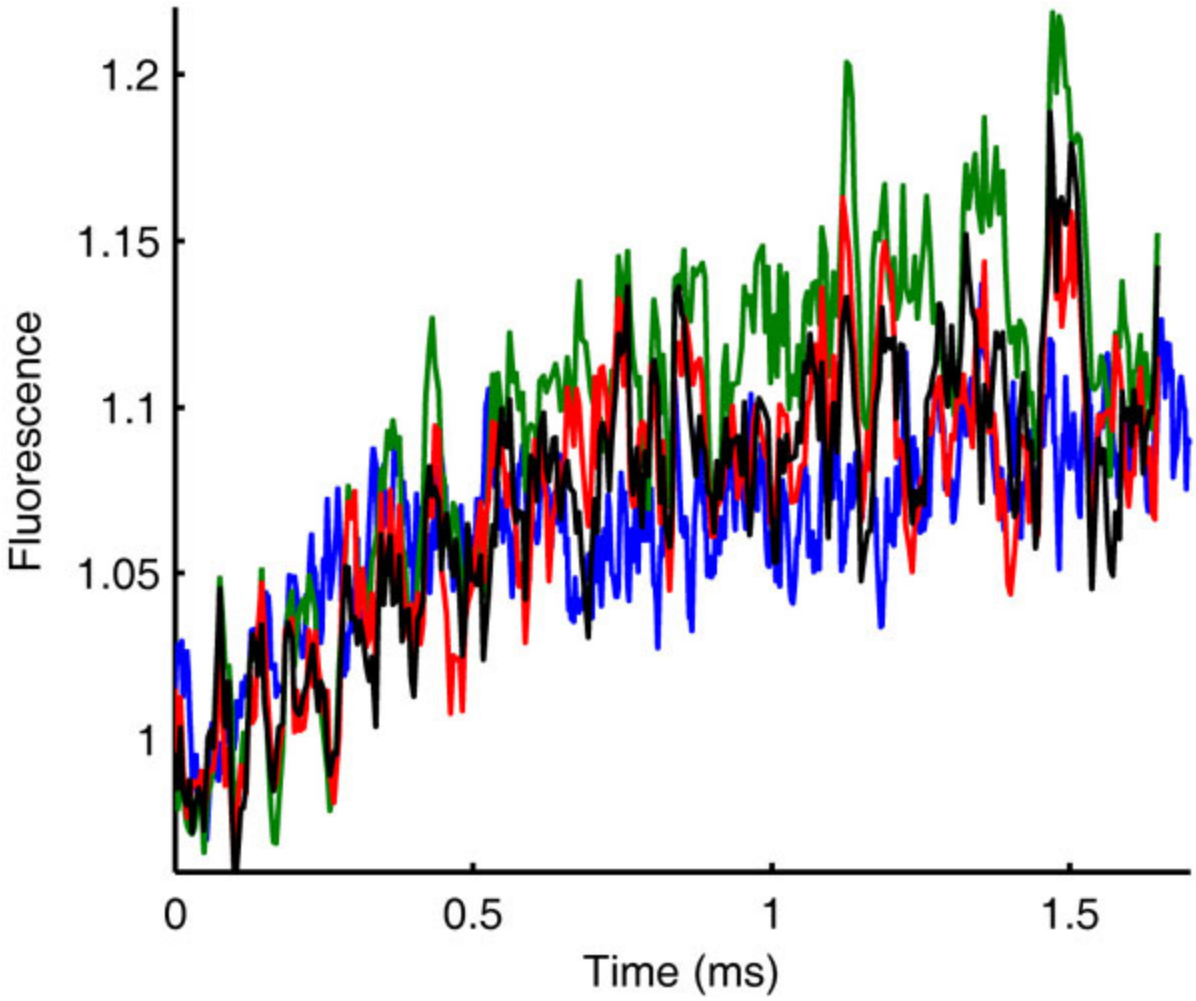

Fig. 9. Kinetics of conformational changes in C-lobe of CaM_{Acr} when mixed with 1 mM $CaCl_2$ (blue line and green line), 3 mM $CaCl_2$ (red line), and 10 mM $CaCl_2$ (black line) in the microfluidic mixer. A single exponential fit to each dataset yields 2,150 ± 150, 2,000 ± 180, 2,190 ± 230, and 1,790 ± 200 s^{-1}, respectively.